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Early embryogenesis of the vestibular apparatus in mammals with different ecologies

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Abstract

This is an extensive comparative study of pre-natal development of the vestibular apparatus in terrestrial, semi-aquatic, and aquatic mammals. The inner ear of mammals, unlike the outer and middle ears, is characterized by a variety of functions, while the structural organization is unvaried. In the cochlear and vestibular organs, mammals species vary in the topography, shape, and sizes of the structures. Some morphological features depend on the stage of differentiation of the sensory epithelium in the inner ear. In terrestrial and semi-aquatic species, the primary cellular differentiation begins in the macula utriculi, whereas in aquatic mammals the macula sacculi is the site of differentiation. Therefore, organ gravitation in terrestrial and semi-aquatic species has a more important function compared to organ vibration, which in aquatic species develops earlier and is vitally important for them. The study of the embryogenesis of the receptor structures of the vestibular apparatus in mammals with different ecological specializations is an important step to understanding commonalities in the development of structures and functions of the inner ear of mammals and contributes to our understanding of the evolutionary origin of the labyrinth among vertebrates.

Key words: cetacean, pinniped, sacculus, utriculus, semicircular canals, vestibular apparatus, cochlea, utricular macula, saccular macula, crista ampullaris, receptor cells, organ of corti, terrestrial, semi-aquatic, and aquatic mammals.

Introduction

The evolutionary origin of the labyrinth among vertebrates so far remains unknown in spite of hypotheses which explain its evolution from the lancelet to mammals. It is well known that the labyrinth originated on the organs of the lateral line, which has direct contact with the environment. An increased complexity in the structures and functions of the organs of the lateral line caused the appearance of a new structure—the vestibular apparatus. However, researchers cannot explain the evolution from an open labyrinth to a closed structure.

The present study investigates this problem through a comparative analysis of the early embryogenesis of the vestibular and cochlear parts of the inner ear in terrestrial, semi-aquatic, and aquatic mammals and demonstrates the scope of the evolutionary transformation mammals underwent in adaptation to life in water.

Several years of investigations of the pre- and post-natal development of the acoustic and vestibular structures in terrestrial animals are available (Held, 1926; Engstrom, *et al.*, 1963; Webster, 1966; Brant, 1970). However, these data are of limited use for understanding the structure-function relationships in the auditory and vestibular apparatus of mammalian species from different ecological groups (i.e., terrestrial, semi-aquatic, and aquatic). Only comparative analysis of embryonic development has the potential for elucidating the range of adaptive transformations of the auditory and vestibular structures in species with different ecological specializations (Bogoslovskaya & Solntseva, 1979).

The vast difficulties in the collection of embryos from marine mammals have resulted in the auditory and vestibular organs of these groups remaining unstudied. I have studied the structural and functional organization of the peripheral auditory system in pre- and post-natal ontogenesis in representatives of the different ecological groups of mammals (Solntseva, 1992; 1993; 1995). Herein, another component of the inner ear, the vestibule, becomes the subject of my investigations. Compared to the auditory function of the cochlea, diverse vestibular functions are of prime importance in most mammals. I conducted comparative embryological studies of the auditory and vestibular structures on unique collections of embryonic organs taken from marine mammals. This study established trends in development of the auditory and vestibular apparatus in mammals (Solntseva, 1996; 1997a; 1997b; 1998a; 1998b; 1999a, 1999b).

Materials and Methods

The following species of mammals were studied: Rodentia—laboratory rat (*Rattus norvegicus*), guinea pig (*Cavia porcellus*); Artiodactyla—pig (*Sus scrofa domestica*); Cetacea—Odontoceti: spotted dolphin (*Stenella attenuata*), common dolphin (*Delphinus delphis*), bottlenose dolphin (*Tursiops truncatus*), harbor porpoise (*Phocaoena phocaoena*), beluga (*Delphinapterus leucas*); Mysticeti: minke whale (*Balaenoptera acutorostrata*); Pinnipedia—Otariidae: Steller sea lion; (*Eumetopias jubatus*); Phocidae: ringed seal (*Phoca hispida*), bearded seal (*Erignathus barbatus*); and Odobenidae: walrus (*Odobenus rosmarus divergens*).

Specimens were fixed in 10% buffered formalin and Wittmaak fixative then dehydrated and treated in an increasing series of ethanols, embedded in celloidin, and sectioned at 10–15 micron thickness in a dorso-ventral plane. The sections were stained with hematoxylin-eosin, according to the methods of Mallory and Kulchitsky, and impregnated with silver nitrate.

The duration of gestation and lengths of embryos at diverse stages of embryogenesis vary widely among mammals. To examine embryos from diverse species, I compared developing structures of the vestibular apparatus with the development of acoustic structures at the same stage of development. For convenience, we used the stages of development described in certain terrestrial species (Dyban *et al*., 1975).

Results

The auditory and vestibular apparatus of mammals are absolutely independent organs characterized by a high anatomical complexity and pronounced multicomponent structure. The mammalian inner ear contains the organ of hearing (organ of corti) and the vestibular apparatus, which is responsible for maintaining body balance. The vestibular apparatus consists of three semicircular canals, which are positioned in three mutually perpendicular planes, and two membranous sacs (round sacculus and oval utriculus). Previous studies showed that semicircular canals respond to angular acceleration, whereas the membranous sacs respond to linear accelerations (Goltz, 1870). Ampullae, the dilations of semicircular canals, contain receptors (crista ampullaris or auditory crests). The ampullae of the semicircular canals are connected with the base of the utriculus. The receptors of the sacculus and utriculus are organized into maculae. The macula utriculi are found in the inferolateral wall of the utriculus, and the macula neglecta in the medial wall of the utriculus and the macula sacculi.

The paired rudiment of the membranous labyrinth is observed at the stage of 2–3 pairs of somites stage of development (Wilson, 1914). At the stage of 6–9 pairs of somites, the rudiment of the membranous labyrinth is the auditory placode (Kappers, 1941). Later, at the stage of 20 pairs of somites (stage 13 of development of the forelimb stage), the auditory vesicle develops and merges with the ductus endolymphaticus (Titova, 1968). In all studied mammals, at stages 14–15 the auditory vesicle is divided into an upper and lower segment. The upper segment gives rise to the utriculus and semicircular canals. From the lower segment, the cochlear duct and sacculus develop (Figs. 1A, 2A, 3A, and 4A).

At the 16th stage, in different species the region in the upper part of the wall of the upper sacculus becomes thick and forms flat pockets whose opposite walls sticks to each other (Figs. 5 and 6). In further development (Fig. 7), the areas of cohesion are resorbed, and semicircular canals are formed from the edges of the pockets (De Buriet, 1934). Both vertical canals are formed from a common bud, and their rear ends are connected with the middle part of utriculus. The opposite ends of semicircular canals go directly to the utriculus and form ampullae (Figs. 1B, 1C, 2B, 3C, 6C and 7).

A comparative morphological analysis showed that the vestibular apparatus of semi-aquatic mammals (otariid seals and walrus) is twice as large as the cochlear segment of the inner ear (Figs. 1D, 5A, 5B and 6), whereas the vestibular apparatus of absolute hydrobionts (cetaceans) is two times smaller than their cochlea (Figs. 2C, 3B, 4B, and 4C) A similar ratio between the sizes of the cochlear and vestibular organs was observed in earless seals (Fig. 6C). In all studied species, the semicircular canals appeared as hollow tubes with well-developed ampulles. The cochlear canal has a clearly distinguishable base formed by cylindrical epithelium and the roof consists of cubic epithelium. At this stage, the cochlear duct begins to wind and forms the basal turn of the cochlea surrounded by the ear capsule, which consists of a dense mesenchyme (Figs. 2A and 3A).

At stage 17 of development, the lumens of semicircular canals, sacculus, utriculus, and crista ampullaris are enlarged in terrestrial and semi-aquatic mammals (Figs. 1B, 1C, 1D, 5B, 5C, 6B, 6C, 7A, 7B and 7C). The sizes of the lumens of the two vertical and one horizontal semicircular canals vary in different species. As in species that spend most of their life on land, the semicircular canals have a large diameter (Figs. 4B, 4C, 5A and 6C). In

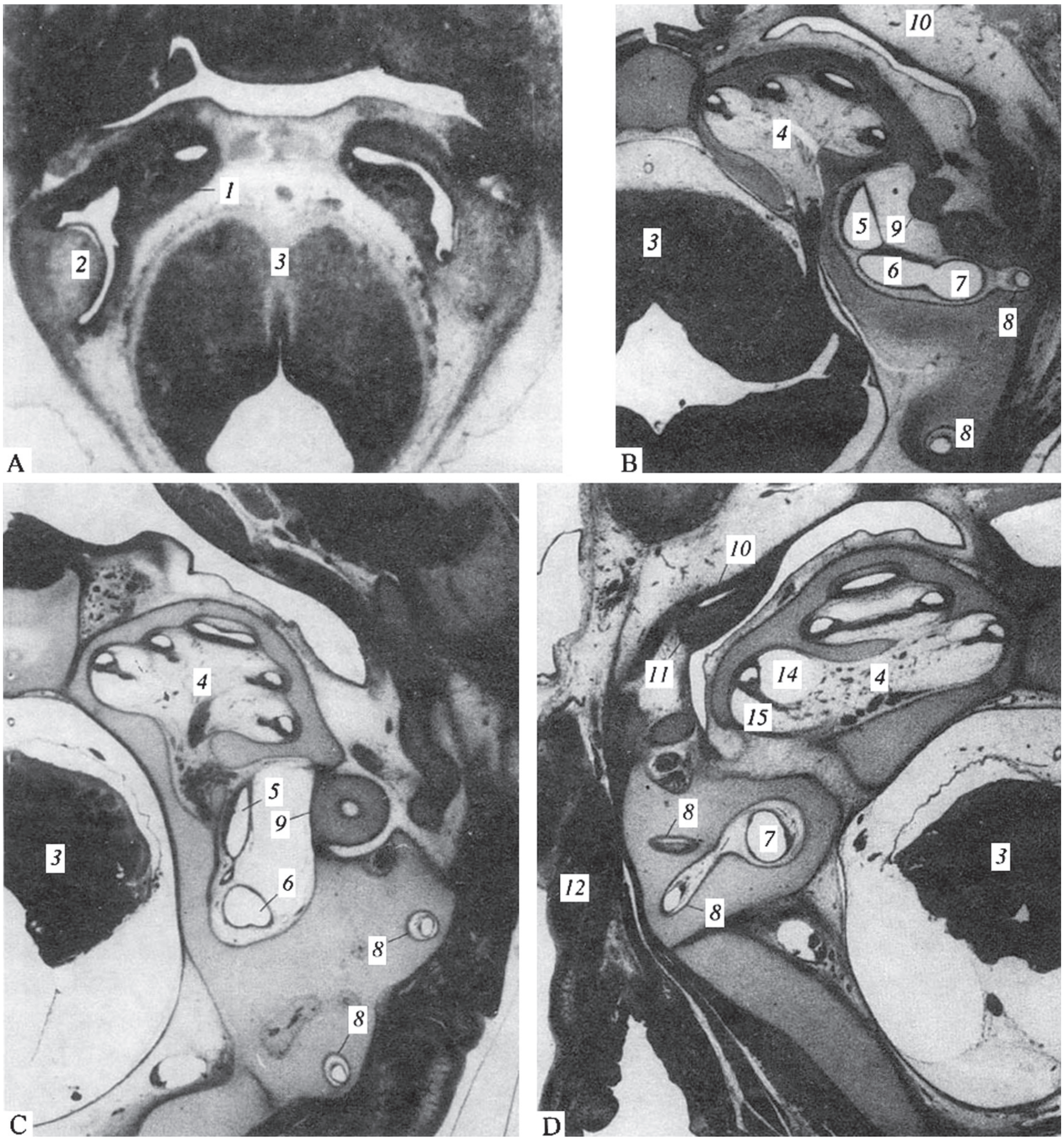

Figure 1. Prenatal development of the auditory and vestibular structures of *Rattus norvegicus* prefetus (A—stages 14–15) and Sus scrofa domestica prefetus (B—stages 17–18; C, D—stages 19–20). Dorso-ventral sections of the head. Labels of all figures (1–7): 1—cochlear canal; 2—vestibular apparatus; 3—cerebrum; 4—cochlea; 5—sacculus; 6—utriculus; 7—ampulla of the semicircular canal; 8—semicircular canal; 9—stapes; 10—tympanum; 11—manubrium of mallei; 12—pinna; 13—musculus stapedius; 14—scala vestibuli; 15—scala tympani; 16—cochlear nerve; 17—vestibular nerve; 18—crista ampullaris; 19—vestibular ganglion; 20—utricular macula; 21—saccular macula; 22—receptor cells of the saccular macula; 23—ear capsule; 24—malleus; 25—cochlear ganglion; 26—incus; 27—external auditory meatus; 28—acoustic nerve; 29—ductus endolymphaticus.

aquatic mammals, the size of the whole vestibular apparatus and individual components (including the semicircular canals) is decreased relative to the cochlear segment (Figs. 2B, 2C, 3B, 3C and 4C). The formation of an ampulla is completed in the vertical semicircular canal, the posterior vertical semicircular canal also is formed, and its lumen increases considerably to adopt a rounded shape.

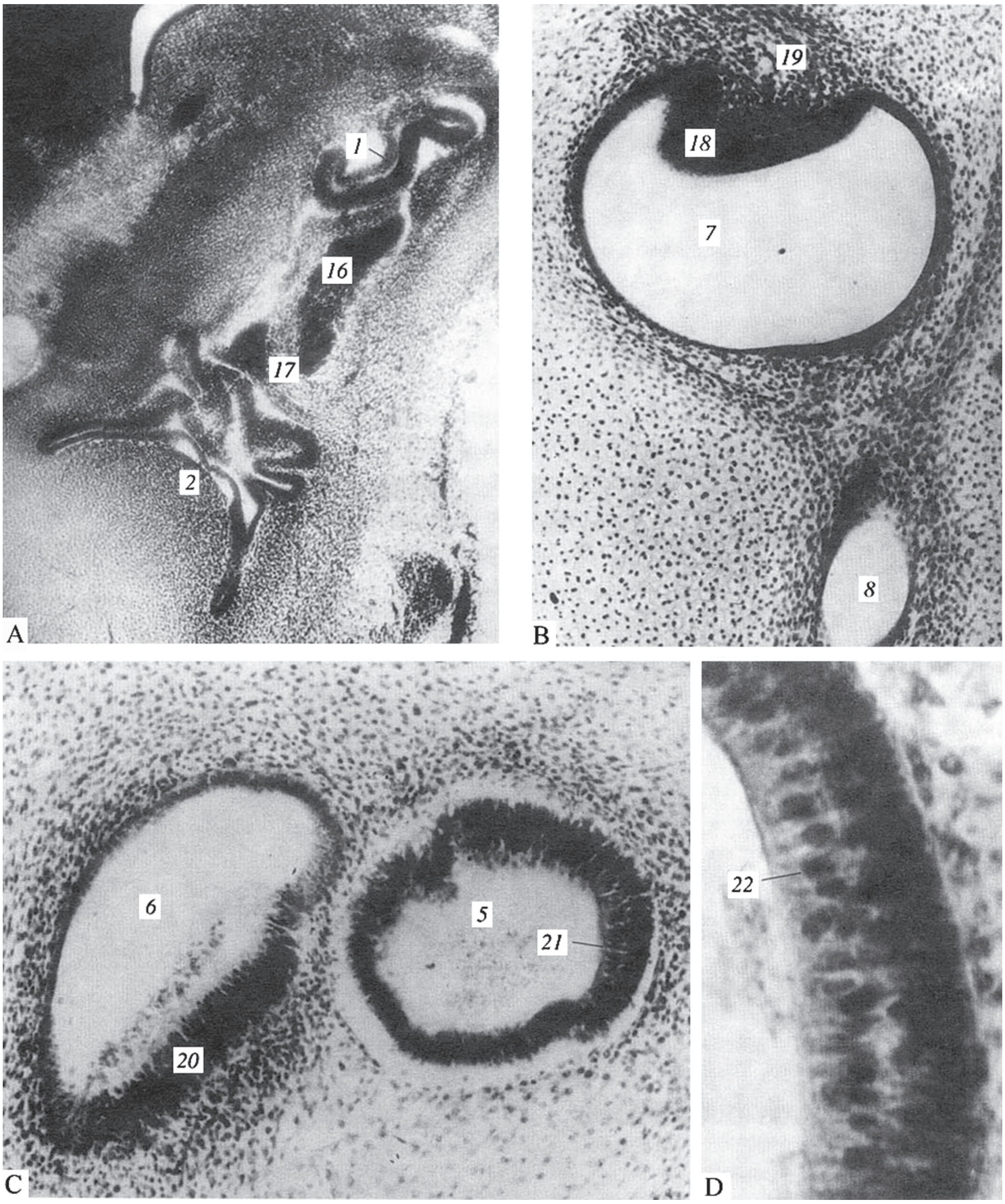

Figure 2. Structure of the vestibular apparates in the dorso-ventral sections of the head of *Stenella attenuata* (prefetus). A—stages 14–15; B , C, D—stages 18–19. Labels as in Figure 1.

In semi-aquatic mammals (otariid seals, walrus), the primary differentiation of the sensory epithelium into the receptor and supporting cells occurs in the utricular macula, which is typical of terrestrial species. In phocid seals, the differentiation of the epithelial cells was not observed in either saccular or utricular maculae. In totally aquatic mammals, cell differentiation occurs

Figure 3. Prenatal development of the auditory and vestibular structures of *Delphinapterus leucas* (prefetus, dorso-ventral sections of the head). A—stages 14–15; B—stages 17–18 ; C, D—stages 18–19. Labels as in Figure 1.

only in the saccular maculae (Figs. 2C, 2D). The cells of the utricular macula are differentiated at later stages, and the cells of ampuliar crests are differentiated even later. In the cochlea, the medial turn was generated. No differentiation into receptor and supporting cells was seen in the organ of corti (Figs. 4B, 5B and 6A).

At the 18th stage, the size of the structures of the inner ear continued to increase in all studied species. The receptor macula of the utriculus adopts a more horizontal position in relation to the saccular macula, whose position is nearly vertical. The two maculae make right angles to each other (Figs. 1B, 2C and 7C). Each macula is an individual organ with its own specific function. The receptor epithelium of the crista ampullaris is similar to the receptor epithelium of the maculae. The sensory epithelium of the crista ampullaris coverts both its crest and the two lateral sides. The ampullae are formed in the anterior vertical and horizontal semi-circular canals; whereas, the formation of ampulla in the posterior vertical semicircular canal is ahead of this process in other canals. In humans, the growth of the horizontal and posterior

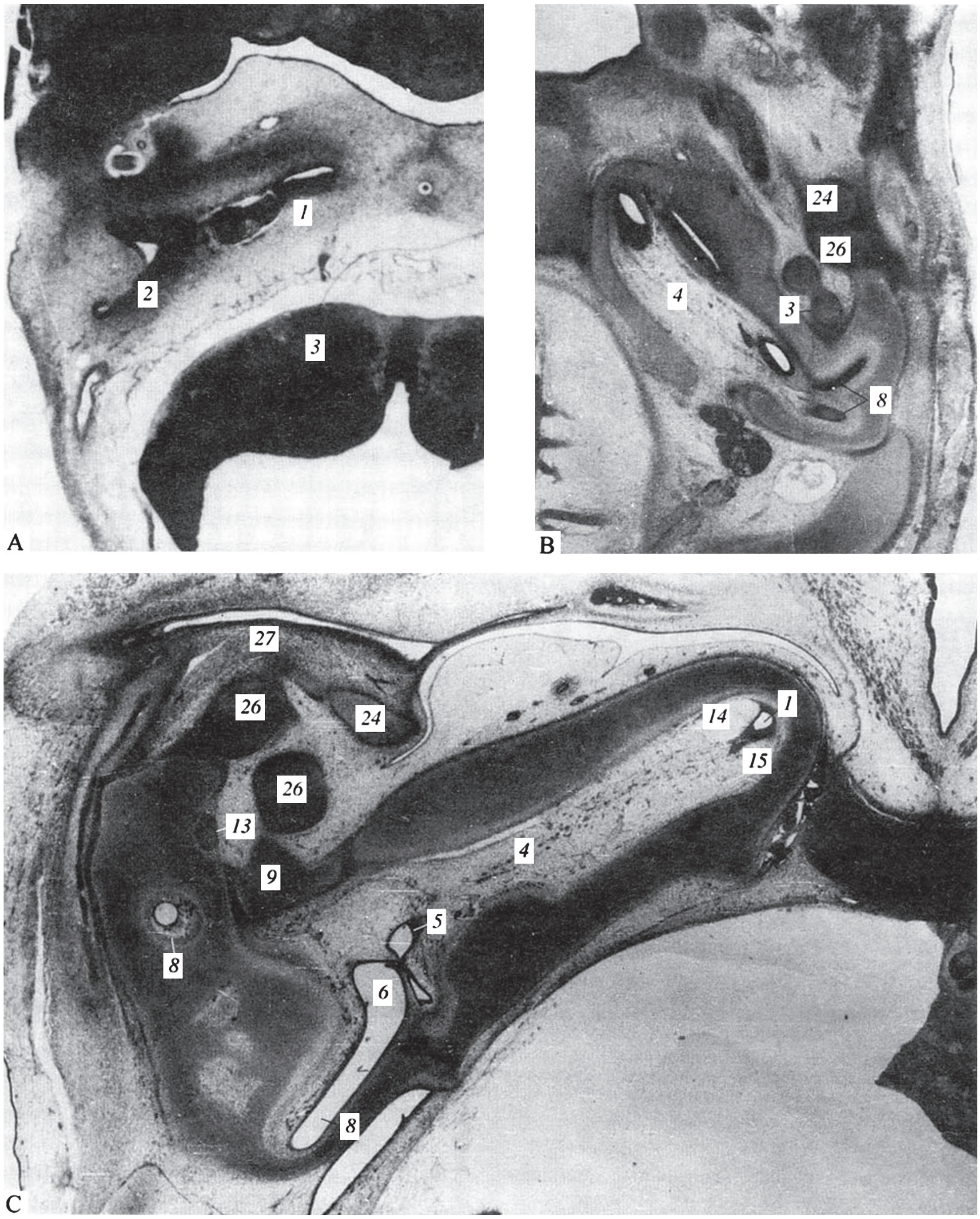

Figure 4. Structures of the cochlear and vestibular segments of the inner ear in the dorso-ventral sections of the head of *Balaenoptera acutorostrata* (prefetus). A—stages 14–15; B—stages 17; C—stages 18–19. Labels as in Figure 1.

vertical semicircular canals is complete by the seventh month of development; whereas, the anterior vertical semicircular canal grows until birth (Gagloeva, 1973). The author of the cited work explained this phenomenon as of greater importance in the anterior vertical semicircular

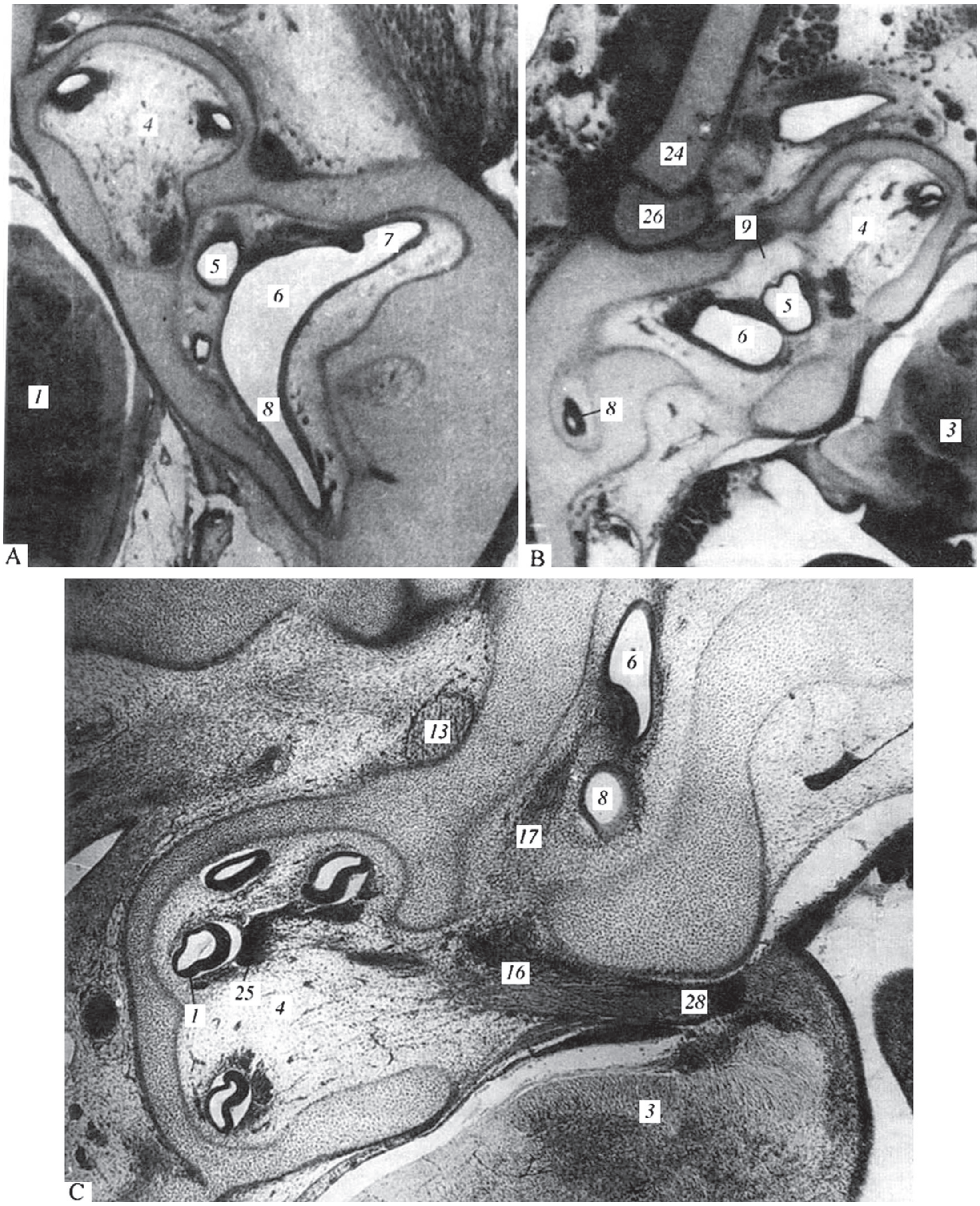

Figure 5. Prenatal development of the cochlear and vestibular structures in the dorso-ventral sections of the head of *Eumetopias jubatus* (prefetus). A—stage 16; B—stage 17; C—stages 18–19. Labels as in Figure 1.

canal compared to the horizontal and posterior vertical canals. This structure is responsible for the vertical position of the body.

The cochlea of most mammals finished anatomical formation by completing the apical turn (Figs. 1C, 1D, 3B and 5C) and the ear capsule

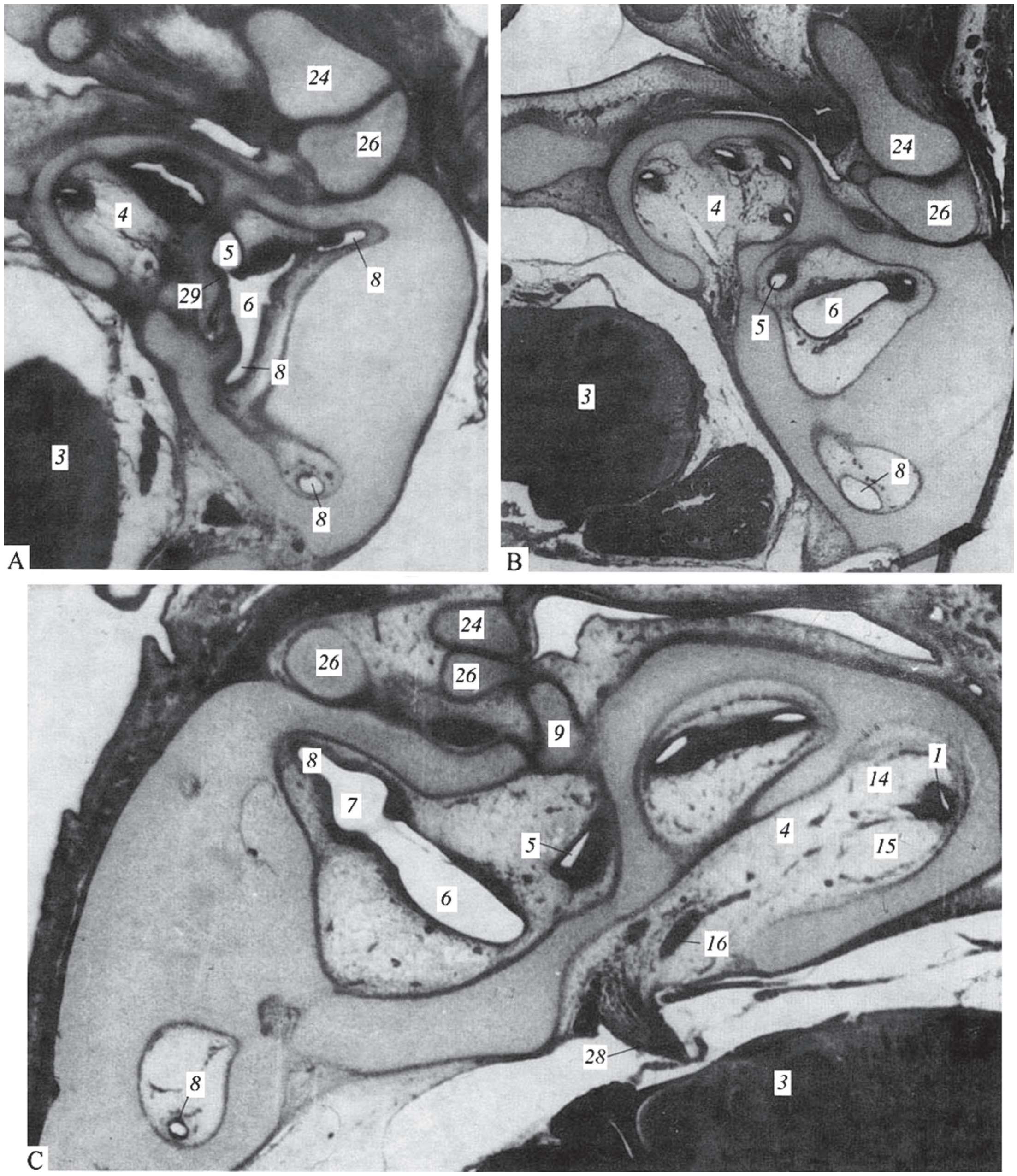

Figure 6. Structures of the cochlear and vestibular segments of the *Odobenus rosmarus divergens* (prefetus, dorso-ventral sections of the head). A—stages 16–17; B—stages 17–18; C—stages 19–20.

becomes cartilaginous. Elements of the organ of corti are at the same stage of cell differentiation as all cochlear turns. At this stage, the cylindrical epithelial cells seem to be drawn apart, and the epithelium forms two (the axial and lateral) dilations.

At the 19th stage of development, in all studied mammals, the dimensions of semicircular canals, sacculus, utriculus, acoustic crests, and ampullar parts of semicircular canals become greater (Figs. 1B, 1C, 1D, 2B, 2C, 3B, 3C, 4C, 5C, 6C and 7C). In terrestrial and semi-aquatic mammals, the

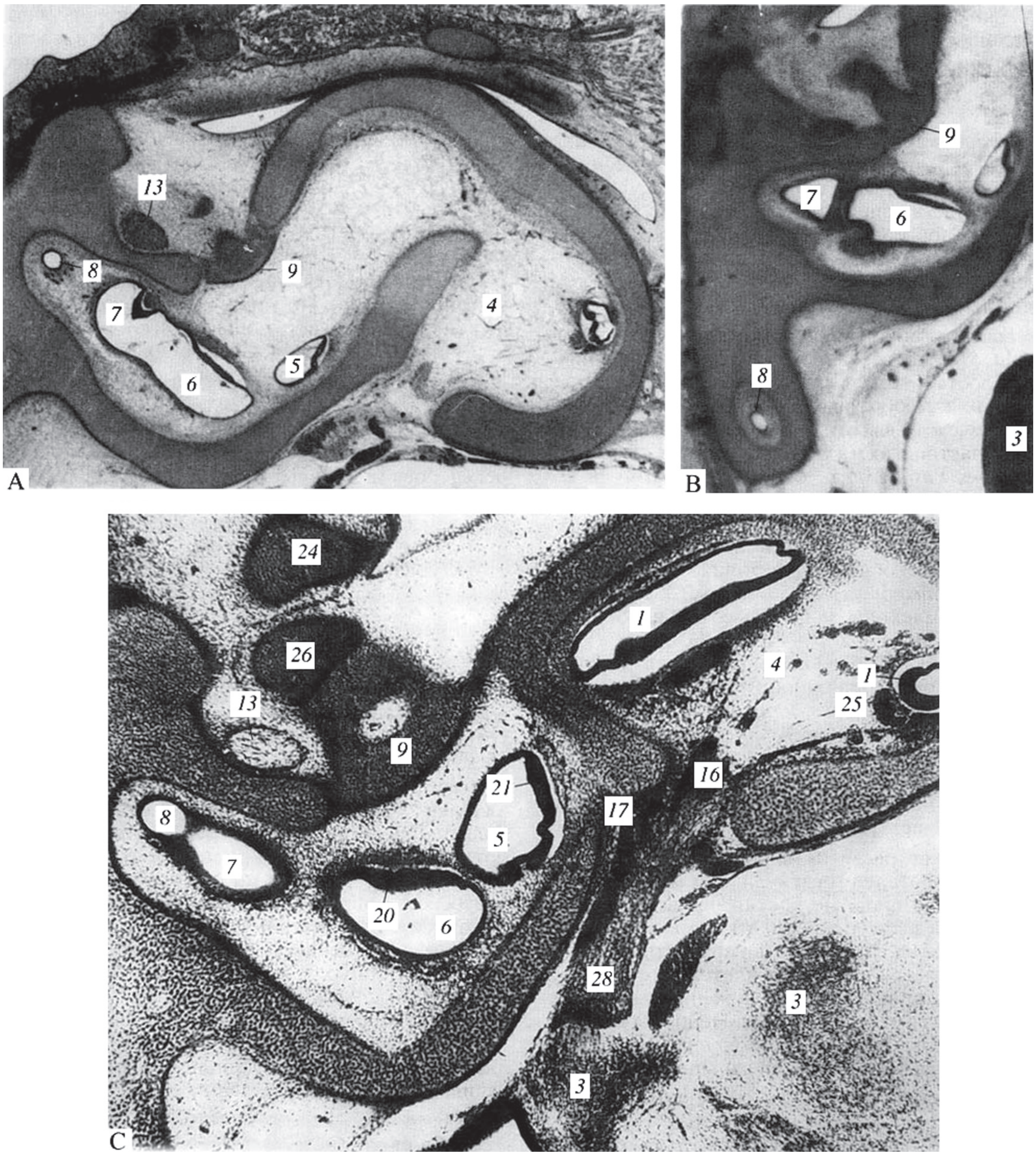

Figure 7. Prenatal development of the cochlear and vestibular structures of *Erignathus barbatus* (prefetus, dorso-ventral sections of the head). A—stage 17; B—stage 18; C—stages 19– 20. Labels as in Figure 1.

sensory epithelium is differentiated simultaneously at several sites in the utricular macula and cristae, thereby covering considerable areas of the organ (Titova, 1968). In aquatic mammals, the differentiation of the saccular macula mainly occurs in the central part of the sensory epithelium. The structures of receptor and supporting cells is clearly visible, and the cells are differentiated. The receptor cells form one row in the upper part of the sensory epithelium (Fig. 2D). The cell body is cylindrical and contains a large oval or round nucleus. The cell nuclei are light. Layers of supporting cells are extended from the basal membrane to the apical surface of the sensory epithelium. The narrow parts of the supporting cells are positioned between the receptor cells. The borders of supporting cells are invisible. Their nuclei are small, dense, and oval. Receptor and supporting cells form a mosaic picture characteristic of the organ of corti. The vestibular and auditory ganglions are well-developed. Its neurons contain large round nuclei in terrestrial and semi-aquatic species. In aquatic mammals, the nuclei are large ovals.

In the cochlea of all studied mammals, the elements of the cochlear duct and the organ of corti are differentiated. The cells of the cubic epithelium are flattened and the adjacent connective tissue is loosened. The vestibular and tympanic scales begin from this site (Figs. 1C, 1D, 4C and 6C). The cells of the organ of corti are differentiated from the cells of the cylindrical epithelium, which seems to be drawn apart. The differentiation of elements of the organ of corti begins from the basal turn of the cochlea and spreads to the upper turns, resulting in different degrees of anatomical differentiation of cells in individual turns.

At the 20th stage of development, in terrestrial and semi-aquatic mammals, the size of the vestibular apparatus relative to the cochlea increases two-fold (Figs. 5C and 6C). In contrast to these species, in aquatic mammals the size of the cochlea relative to vestibular apparatus increases another two-fold (Figs. 3B and 4C). Semicircular canals, sacculus, utriculus, and ampullar crests are all enlarged. The sensory epithelial cells of the ampullar crests and saccular macula in terrestrial and semi-aquatic species begin differentiation. The aquatic mammals begin differentiated ampullar crests and utricular macula. However, the differentiation of the sensory epithelium of crests and the utricular macula is delayed relative to the sensory epithelium of the saccular macula.

Although the vestibular apparatus of most species consists of identical components, the structure of the components varies among species. This particularly concerns topography, shape, and size of the vestibular structures.

The cochlea is anatomically formed in all studies species. The spiral limbus, stria vascularis, and the techorial and Reissner's membranes differentiate in the cochlear duct. Vestibular and cochlear branches of the acoustic nerve are well-developed (Figs. 5C, 6C and 7C). Elements of the cochlear duct and organ of corti continue to differentiate.

Discussion

This comparative embryological study showed that in mammals with different ecologies, the formation of the inner ear structures proceeds with a similar sequence and roughly at the same developmental stages, i.e. during the formation of the cartilaginous skeleton, from the formation of the acoustic vesicle (stage 13) to the completion of all anatomical structures of the cochlear and vestibular organs (stages 20 to 21). The cochlear and vestibular structures are separated from each other at an early embryonic period. However, the formation of cochlear and vestibular structures in terrestrial, semi-aquatic, and aquatic mammals takes more time because of heterochronous development of the inner ear.

In the representatives of mammals from different ecological groups there are general regularities in the development of the outer, middle, and inner ears:

(1) in the first half of the early ontogenesis, the auditory and vestibular structures (stages 13 to 15) most mammals share common structural features;
(2) in the structural organization of the auditory and vestibular organs, species-specific features are formed in the second-half of the early embryogenesis (stages 16 to 20), depending on ecological specialization of the animal;
(3) cell differentiation of the inner ear continues to develop in later embryogenesis, fetal, and early postnatal periods;
(4) in aquatic mammals, cell differentiation of the auditory and vestibular structures is completed by birth.

This study revealed that some morphological features vary with stages of the differentiation of the sensory epithelium in the structures of the inner ear. In terrestrial and semi-aquatic mammals (otariidae, odobenidae); primary cell differentiation occurs in the utricular macula. Organ gravitation in these species has a more important function than organ vibration. The simultaneous cellular differentiation of the sensory epithelium in utricular and saccular maculas and similar sizes in earless seals support the notion that both organs of gravitation and vibration are vitally important. In aquatic mammals (cetaceans), an early differentiation of sensory epithelium into receptor and supporting cells is found in the saccular macula; well before the beginning of cytological differentiation of the utricular macula and ampullar crests. The early differentiation of sensory epithelium of the saccular macula in cetaceans suggests that the organ of vibration (sacculus) performs a more important function that the organ of gravitation (utriculus).

In terrestrial and semi-aquatic mammals, the differentiation of the sensory epithelium of the organ of corti is delayed relative to the sensory epithelium of the saccular and utricular maculas. In aquatic mammals, the differentiation of the organ of corti is practically completed by the time of birth. In the immature-born species, differentiation of the elements of the cochlear canal and the cells of the organ of corti is not completed until the 20th day of the early postnatal ontogenesis.

In this way, the outer and middle ears are characterized by various additional structures for life under water and the inner ear among mammals was less variable. At the monotonous structure organization the functions of the vestibular apparatus can be very different, whereas the peripheral auditory structures have different structural organization with the only an auditory function.

In the cochlear and vestibular organs, all mammals changed the topography, shape, and sizes of different structures. A significant increase in the cochlear size in aquatic mammals compared to the size of its vestibular apparatus is accounted for by the fact that the cochlea is more closely related to the auditory function than other structures of the inner ear. Transmitting acoustic information in aquatic mammals serves a vital role in their navigation, communication, and echolocation (in some species). The study of the embryogenesis of the receptor structures of the vestibular apparatus in mammals with different ecological specializations is a step to understanding important regularities of the development of the structure and functions of the inner ear of all mammals.

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